Grepafloxacin: microbiological properties

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Grepafloxacin is a new broad-spectrum fluoroquinolone characterized by having a methyl-substituted piperazine at the 7 position. It is a water-soluble racemate with both stereoisomers having the same activity. Its mode of action involves inhibition of topoisomerases II and IV. Grepafloxacin is not recognized by the NorA efflux mechanism in *Staphylococcus aureus* and, thus, some strains of *Staphylococcus aureus* that are resistant to other fluoroquinolones remain susceptible to grepafloxacin.

Grepafloxacin has potent in vitro activity against streptococci and staphylococci, respiratory Gram-negative pathogens, atypical respiratory pathogens and sexually transmitted disease pathogens. It combines the positive properties of the β-lactams against conventional Gram-positive and Gram-negative respiratory pathogens with the activity of the macrolides against the atypical pathogens. Unlike macrolides, it is bactericidal at concentrations close to the MIC. Its in vitro activity has been reflected in animal models of respiratory tract infections. Concentrations above MICs are maintained throughout nearly all of the 24-h dosing interval. Grepafloxacin provides important improvements over older quinolones and over other classes of antibiotics.

Key words: Grepafloxacin, fluoroquinolone resistance, *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis,* atypical respiratory pathogens

MODE OF ACTION

Grepafloxacin is a water-soluble fluoroquinolone, characterized by a methyl group at the 5 position a methyl-substituted piperazine at the 7 position (Figure 1), which provides some of its unique properties [1]. In common with other fluoroquinolones, grepafloxacin's primary target in most organisms is topoisomerase II (DNA gyrase). It is a selective inhibitor of this enzyme, with up to 1000-fold selectivity compared to the mammalian enzyme [2,3] (Table 1). Preliminary evidence shows that fluoroquinolone targets in *Streptococcus pneumoniae* differ according to the compound and that the topoisomerase II and IV enzymes are mediated by the *gyrA/B* and *parC/E* genes respectively. Thus, like sparfloxacin, grepafloxacin may also target topoisomerase II, unlike ciprofloxacin, which primarily targets topoisomerase IV [4].

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Grepafloxacin is a racemate, but the stereoisomers are equally active and their safety profiles do not differ (Glaxo Wellcome, data on file). Thus, there is no advantage in separating the two isomers.

FLUOROQUINOLONE RESISTANCE

Grepafloxacin is not recognized by the NorA efflux mechanism in *Staphylococcus aureus* (Hooper, this supplement). Thus, organisms or strains of *Staphylococcus aureus* that are resistant to quinolones as a result of the NorA efflux mechanism remain susceptible to grepafloxacin.

Pan and Fisher have isolated two series of *Streptococcus pneumoniae* mutants; the C series, which is resistant to ciprofloxacin, and the S series, which is resistant to sparfloxacin. They found that there was a two-step mutation process [4]. With ciprofloxacin, a modest increase in MIC was associated with a mutation in the *parC* gene—i.e., in topoisomerase IV—while the second step, which gave a much enhanced increase in the MIC, was due to a second mutation in the DNA gyrase gene, *gyrA*. Conversely, when the mutants were selected on sparfloxacin, the first-stage mutation—giving a modest increase in MIC—was due to a mutation in the DNA gyrase gene, and a double mutation gave a large increase in MIC (or increase

Table 1 Inhibition of bacterial [2] and calf [3] topoisomerase II-induced DNA supercoiling by grepafloxacin and other fluoroquinolones

Organism	Drug	MIC (mg/L)	IC_{50} (mg/L)
Staphylococcus aureus SA113	Grepafloxacin	0.05	23.0
	Ciprofloxacin	0.4	20.5
	Ofloxacin	0.4	27.0
	Norfloxacin	0.8	91.5
Escherichia coli KL-16	Grepafloxacin	0.025	0.19
	Ciprofloxacin	0.0125	0.11
	Ofloxacin	0.05	0.48
	Norfloxacin	0.05	0.33
Calf	Grepafloxacin	–	>100
	Ciprofloxacin	–	>100
	Ofloxacin	–	>400
	Norfloxacin	–	>100

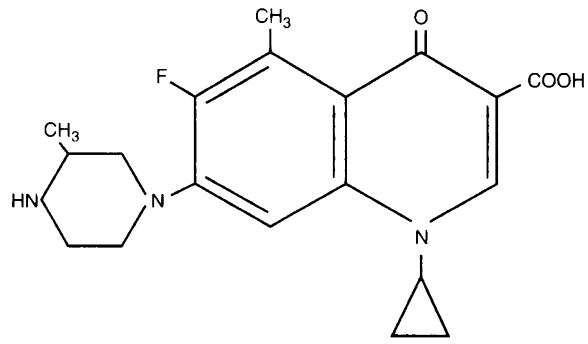

Figure 1 The structure of grepafloxacin, a water-soluble fluorinated quinolone.

in resistance). Fisher's interpretation is that in *Streptococcus pneumoniae*, unlike other bacteria, the primary target for ciprofloxacin is topoisomerase IV, while for sparfloxacin the primary target is DNA gyrase.

We have tested the activity of grepafloxacin against *gyrA* and *parC* mutants kindly provided by Fisher. There is no significant increase in MIC with either a *parC* or *gyrA* mutation. A two-step mutation is needed for high-level resistance to grepafloxacin to be acquired (MIC increase greater than 32-fold). It has been concluded that both *parC* and *gyrA* are equally sensitive to grepafloxacin, and this may explain why it was found impossible, at least with any reasonable frequency, to isolate mutants resistant to grepafloxacin [5]. Mutants resistant to ciprofloxacin could be isolated, but these were not resistant to grepafloxacin. The generation of mutation from single-step mutants to full resistance to grepafloxacin is currently being investigated.

MICROBIOLOGICAL ACTIVITY

Activity against respiratory tract infections

Grepafloxacin is being developed predominantly for the treatment of community-acquired respiratory tract infections. Some studies have been performed in sexually transmitted diseases, but the focus of this paper is on the activity relevant to respiratory tract pathogens.

Grepafloxacin has good activity against *Streptococcus pneumoniae* [6–8] and *Staphylococcus aureus* [9], the Gram-negative pathogens, *Haemophilus influenzae* [8,10] and *Moraxella catarrhalis* [9], and also against the atypical respiratory pathogens [11–13] (Table 2). It is active against Gram-negative organisms, including *Escherichia coli*, *Klebsiella* and a range of other enterobacteriaceae (Table 3) [9,11,14,15]. The activity against *Pseudomonas* is variable [9].

Grepafloxacin has poor activity against anaerobes [16], a characteristic which provides some potential advantages in terms of lack of effect on fecal flora.

Gram-positive organisms

Studies carried out in the USA and Japan on large numbers of strains of Gram-positive pathogens show similar results with respect to MIC_{90}s. The compound has good activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and some other streptococci, but it is not very active against the enterococci [17]. The MICs for grepafloxacin against *Streptococcus pneumoniae* are, on average, about eight-fold lower than for ciprofloxacin and considerably lower than for fleroxacin (Figure 2) [7].

Penicillin-resistant pneumococci

Penicillin-resistant pneumococci are becoming increasingly prevalent worldwide [18]. The frequency

Table 2 Microbiological activity of grepafloxacin against common respiratory pathogens

Organism	MIC_{90} (mg/L) Grepafloxacin	Ciprofloxacin
Gram-positive pathogens		
Streptococcus pneumoniae [8]	0.25	2
Staphylococcus aureus [9]	0.12	1.0
Gram-negative pathogens		
Haemophilus influenzae [8]	0.008	0.015
Moraxella catarrhalis [9]	0.015	≤0.03
Intracellular respiratory pathogens		
Mycoplasma pneumoniae [11]	0.25	2.0
Chlamydia pneumoniae [12][a]	0.06[a]	1.0[a]
Legionella pneumophila [13]	≤0.016	0.03

[a] These values are MIC, not MIC_{90} as only one strain was tested.

Table 3 Activity of grepafloxacin against other microorganisms

Organism	MIC_{90} (mg/L)	
	Grepafloxacin	Ciprofloxacin
Enterobacteriaceae		
Escherichia coli [9]	0.03	≤0.03
Klebsiella pneumoniae [9]	0.25	0.25
Pseudomonas aeruginosa [9]	4.0	2.0
STD pathogens		
Neisseria gonorrhoeae [14]	0.008	0.008
Ureaplasma urealyticum [11]	2.0	16
Chlamydia trachomatis [15]	0.12	-
Anaerobes		
Bacteroides fragilis [16]	8.0	16

distribution of grepafloxacin MICs has been studied against 698 isolates of *Streptococcus pneumoniae* that had been characterized as either penicillin susceptible, intermediate or highly resistant [7]. Over 90% had a grepafloxacin MIC of 0.25 mg/L or below, irrespective of their penicillin sensitivity. This finding has recently been confirmed with fresh clinical isolates from Europe [6,19].

Gram-negative organisms

The activity of grepafloxacin against Gram-negative organisms is comparable to that of ciprofloxacin against *H. influenzae, M. catarrhalis* [8] and the Enterobacteriaceae, e.g. *Klebsiella pneumoniae* and *Enterobacter cloacae* [9]. Ofloxacin is slightly less active against these organisms [2,9]. β-Lactamase-resistant and -sensitive strains of *H. influenzae* and *M. catarrhalis* are equally susceptible to grepafloxacin [8].

Intracellular respiratory pathogens

There is good activity of grepafloxacin against the intracellular or atypical pathogens, *Mycoplasma, Chlamydia* and *Legionella* spp. A recent study has shown that five isolates of *Chlamydia pneumoniae* were sensitive to grepafloxacin, which had a mean MIC of 0.12 mg/L [15]. *C. trachomatis* was also very sensitive to grepafloxacin, with an MIC_{90} of 0.06 mg/L [15].

There are published data on the activity of grepafloxacin against 104 isolates of *Legionella* spp., mainly *Legionella pneumophila* (97 strains); grepafloxacin showed excellent activity, with an MIC_{90} of 0.015 mg/L (range 0.008–0.03) [15].

Effects of serum, inoculum, pH, CO_2, Mg^{2+} and postantibiotic effect

Grepafloxacin is bactericidal at a concentration more or less equivalent to the MIC [20], and this activity is not influenced by the presence of 50% serum [14]. It is rapidly bactericidal [20] and relatively weakly protein bound, and no increase in MIC in the presence of serum would be expected. There is a small inoculum effect. A 10^4 increase in inoculum size results in a doubling of the MIC [20]. The effects of pH and CO_2 on activity were minimal, but magnesium supplementation reduced activity 2–4-fold, in common with other quinolones [20]. Grepafloxacin has a postantibiotic effect, with suppression of growth continuing for 1.6–2.5 h with *Escherichia coli, Enterococcus faecalis, P. aeruginosa* and *Staphylococcus aureus* following removal of the antibiotic [21].

ACTIVITY IN ANIMAL MODELS

Grepafloxacin has been studied in a range of animal models of infection. In a murine model of pneumonia induced by *Streptococcus pneumoniae*, untreated control

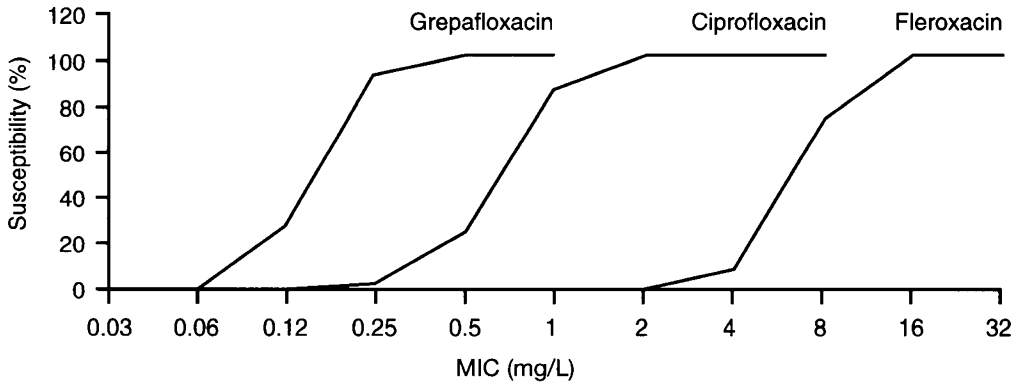

Figure 2 Cumulative susceptibility of *Streptococcus pneumoniae* to quinolones. Adapted from Fuchs et al [7].

animals had about 10^8 bacteria per lung [22]. Grepafloxacin, at 8 mg per mouse, completely eliminated the bacteria and sterilized the lung, in contrast to ofloxacin and ciprofloxacin (Figure 3).

In a model of *H. influenzae* lung infection in neutropenic rats, untreated control animals had about 10^5 organisms per lung. A single dose of either 1 mg/kg or 2 mg/kg of grepafloxacin caused an almost three-log reduction in the number of organisms in the lung (Figure 4) [23]. Grepafloxacin is more active in this model than ciprofloxacin and ofloxacin, which were substantially less effective at reducing bacterial load.

ACTIVITY IN HUMANS

Figure 5 shows the mean steady-state plasma levels after two doses of grepafloxacin, 400 and 600 mg, in male volunteers [24]. The MIC_{90}s for the pathogens *Streptococcus pneumoniae*, *H. influenzae*, *C. trachomatis* and *M. catarrhalis* are shown. Even at 400 mg, the plasma concentration of grepafloxacin remains above the MIC_{90} for *Streptococcus pneumoniae* for almost the whole of the dosing interval. The predictive value in clinical efficacy of the pharmacokinetic/pharmacodynamic parameters is discussed in the paper by R. Wise in this supplement.

SUMMARY

Grepafloxacin's antibacterial spectrum encompasses the Gram-positive and Gram-negative coverage of the second-generation cephalosporins and the atypical coverage of the macrolides with bactericidal action in a once-daily dosage regimen. Thus, grepafloxacin is active against all the common bacterial pathogens responsible for community-acquired respiratory tract infections. It is bactericidal, and its activity is not

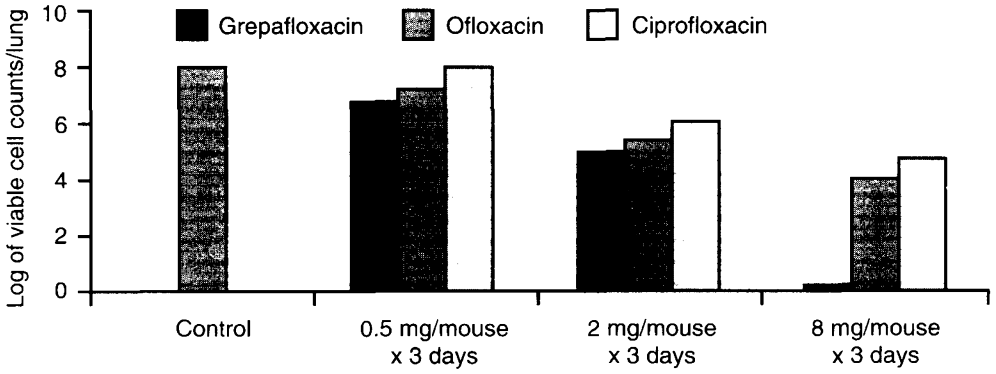

Figure 3 Effect of grepafloxacin and other quinolones in a murine model of pneumonia caused by *Streptococcus pneumoniae* ($n=6$ in each group). Based on Wakebe H. et al [22].

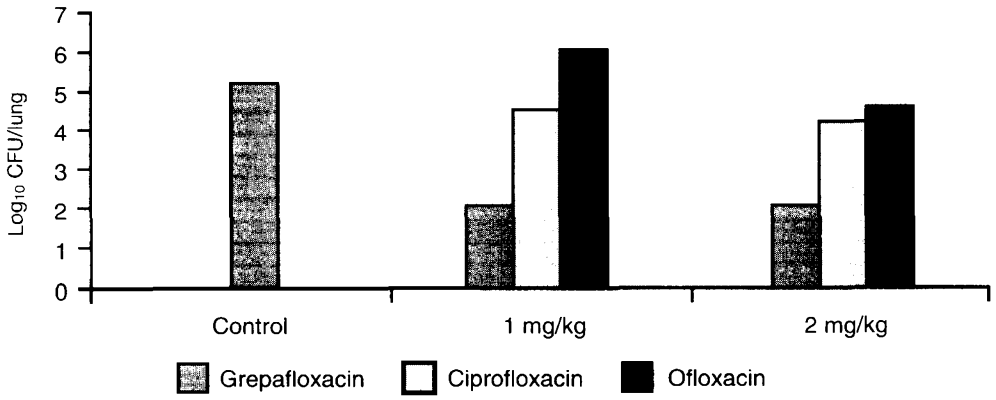

Figure 4 Activity of grepafloxacin and other quinolones in a neutropenic rat lung infection induced by *Haemophilus influenzae*. Based on Ohmori et al [23].

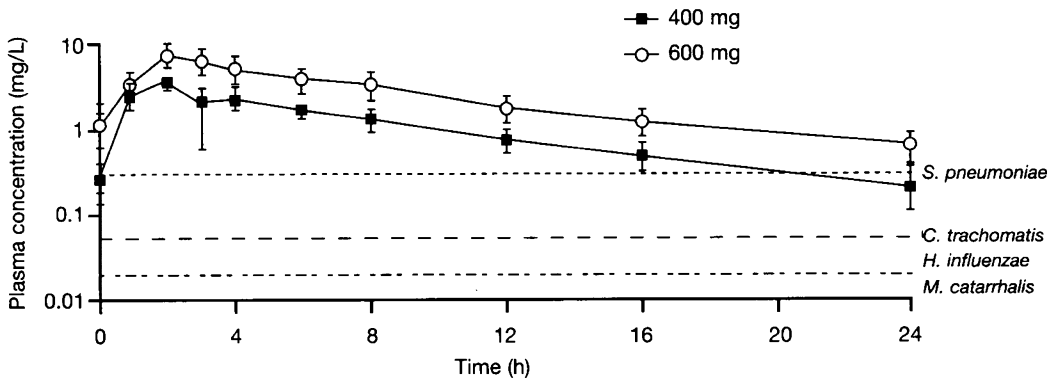

Figure 5 Comparison of mean steady-state plasma levels of grepafloxacin, 400- and 600-mg doses, in male volunteers with MIC_{90} values of pathogens of interest. Based on Efthymiopoulos et al [24].

affected by the presence of serum or plasma proteins. There is a marginal inoculum effect, and little effect with pH, CO_2 or magnesium supplementation. There is a postantibiotic effect which is similar to that observed with other quinolones [21].

The activity of grepafloxacin against conventional Gram-positive and Gram-negative respiratory pathogens is equivalent to that of a β-lactam antibiotic. However, penicillin-resistant pneumococci are equally susceptible to grepafloxacin, as are β-lactam-resistant strains of *H. influenzae* and *M. catarrhalis*. In common with the macrolides, grepafloxacin is highly active against atypical respiratory pathogens. The properties of this compound are ideally suited for the treatment of community-acquired respiratory tract infections.

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